



# ANIMAL TRACKS

A newsletter for the Duke research community



October 2009

<http://vetmed.duhs.duke.edu>

## ANNUAL ANIMAL NUMBERS REPORT

In a few weeks all researchers using animals in research, testing, or teaching will receive an email requesting they report the numbers of animals used this past year. This is a TIME SENSITIVE report to the federal agencies; it is critical that the institution respond by the deadlines, which requires timely reporting from each of our researchers who use animals in their work. The 'request by' date will be November 9th.

The reporting period is from October 1, 2008 thru September 30, 2009. The report includes all animals purchased, bred, or used for all manners of research. The categories of reporting are:

**Category B:** The number of animals being bred, conditioned, or held for use (not yet used); but not reported in Category C, D, or E. (Breeding, conditioned animals not yet euthanized as of September 30, 2009)

**Category C:** The number of animals used in procedures not causing pain, distress and not requiring the use of pain relieving drugs (Examples: AVMA approved methods of euthanasia, non-surgical procedures requiring brief restraint, behavioral observations, and field observations).

**Category D:** The number of animals used in procedures which could be painful, but for which appropriate anesthetic, analgesic, or tranquilizing drugs were used (Examples: Any use of anesthesia or analgesia such as surgery or invasive studies).

**Category E:** The number of animals used in procedures producing pain or distress for which the use of an anesthetic, analgesic or tranquilizing drug would adversely affect the procedures, results, or interpretation of the scientific purpose of the experiment.

Please begin considering what data you will need to draw together to make the necessary reporting deadline.

Best wishes for a productive research week,

## ANNUAL TRAINING FOR ANIMAL USERS

A few years ago, the Duke animal program began a Compliance Liaison (CL) program to assist PIs with remaining compliant to approved animal activities, with a secondary goal of decreasing risk to the researcher and the institution from reportable adverse events. The Duke CL process has been highly successful in many regards—we have seen the significance of potential adverse events decline and the overall repeatability lessen. Early recognition of developing problem areas, and quick corrective actions have prevented most minor and manageable problems from becoming significant adverse events.

The Duke IACUC has determined that tracking and trending of potential non-compliant situations could provide focused information and also address federal encouragements to develop on-going training venues. The result is a brief, current, and practical training refresher rather than the old 'train everyone to everything' approach.

After 2 years of CL activity, the IACUC believes the programmatic trends are now clear and the items we need to revisit annually are becoming defined. It is important to know that this annual training module will be re-visited approximately quarterly, and adjustments will be made in the training slide to remain focused on developing adverse event trends in the animal program.

Beginning in January 2010, as part of the Annual Progress Report, all personnel listed on the approved protocol must complete the Animal Program Annual Update. Hosted on the OESO website and designed to be similar to other institutional training, this module will consist of 10-12 slides with a brief quiz. The goal of the IACUC is to provide approximately 30 minutes of refresher and reminder to those of us using animals in research, testing, or teaching.

For those who wish to accomplish the web training early, stay tuned for details on the training web url.

## ~ IACUC Tip Sheet ~ Maintenance of Guillotines used for Euthanasia Decapitation

While not a common tool for euthanasia, the use of a guillotine for decapitation of small mammals is an acceptable method of humane euthanasia. However, improper use of this device may cause significant pain or distress in animals. The requirements for those situations where a guillotine is required are as follows:

- ◆ **MUST** be IACUC approved prior to use
- ◆ All personnel **MUST** be properly trained and proficient in its use.
- ◆ All animals **MUST** be sedated or anesthetized before decapitation unless precluded by scientific considerations and specifically justified by IACUC approval.
- ◆ Guillotines **MUST** be kept clean and rust-free.
- ◆ Guillotines **MUST** be sharpened at least every 12 months, or more often as needed.
- ◆ Guillotines **MUST** be lubricated as needed with silicone.
- ◆ Operators **MUST** ensure that the action is smooth with no perceptible binding or resistance.
- ◆ The blade **MUST** decapitate with minimal force.

To help lessen the distress or difficulty associated with using guillotines, plastic cones, such as Decapicones™ are strongly recommended. These cones provide good restraint, minimize distress, improve positioning, and enhance operator safety.

Suggested daily maintenance includes:

- ◆ Rinse the entire guillotine under fast-running cold water to remove any blood and tissues,
- ◆ Scrub the base with disinfectant to reduce gross contamination,
- ◆ Perform final rinse with alcohol to ensure evaporation and reduce the need to hand-dry the equipment,
- ◆ Turn the guillotine upside down with the blades opened to facilitate drying.

**NOTE:** Use of scissors (see exception below), knives, scalpels or cutting devices other than a guillotine to perform decapitation is not permitted without approval by the IACUC.

**Exception:** The exception to this rule is when euthanizing rodent neonates (less than 15 days of age). Sharp scissors are acceptable for decapitation.

## ~ IACUC Tip Sheet ~ IACUC Semi-Annual Inspection 'Secrets' Revealed

**Anesthetic Vaporizers:** Do you use an anesthetic vaporizer? Has it been calibrated? Common questions asked during an IACUC Semi-Annual Inspection.

Anesthetic vaporizers must be calibrated either according to the manufacturer's recommendation (a copy of the manufacturer's guidance must be available for the IACUC inspection team), or must be calibrated annually (if no manufacturer's guidance is available).

If your laboratory has a vaporizer that may need calibrating, contact the DLAR Veterinary Services (684-2797) for a list of vendors that provide calibration services. Don't let your lab get caught in a 'gas'!

## ~ IACUC Tip Sheet ~ Reporting Adverse Animal Conditions

**Question: Who is responsible for the condition of Duke owned animals?** Answer: All of us!!!

If you observe an animal in distress, as a member of the Duke animal care program you are obligated to report the distress immediately! You are encouraged to take actions to prevent any further distress to the animal if you can. To report adverse animal conditions, contact the Duke Animal Welfare Hotline (919.684.3535) or Email the Duke IACUC at [IA-CUC@duke.edu](mailto:IA-CUC@duke.edu)

Duke University will not tolerate any misuse or neglect of animals.

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## Effective Monitoring and Recovery of the Anesthetized Rodent

Anesthesia is the act of rendering the patient senseless to pain or discomfort. Anesthesia is used for surgical procedures as well as non-surgical procedures. Assuring a safe and effective level of anesthesia is necessary for quality research and humane use of animals. Assuring a safe and effective level of anesthesia requires monitoring.

According to Duke animal program policy, ALL anesthetized animals must be observed and monitored to assess adequate level of anesthesia, and assure the animal is anesthetized. Failure to monitor properly, especially if the animal experiences adverse outcomes from anesthesia, could be a non-compliance reportable to the federal regulatory agencies (the NIH, the USDA, and AAALAC).

There are as many acceptable methods to monitor anesthesia as there are species of animals, but select monitoring processes are fairly common between species. These include:

**Toe pinch:** Effective if the animal has a toe large enough to pinch. A gentle pinch at or near the nail bed, a pinch which does not break the skin or cause any deep tissue damage, is sufficient to show if the animal has inadequate anesthesia. Any observed movement (e.g. withdrawing the paw) indicates that the animal is not sufficiently anesthetized to perform a painful activity (e.g. surgery).

**Skin pinch:** Similar to the toe pinch but using any skin on the body. More sensitive areas of skin work best. A gentle pinch of a small fold of skin, which does not break the skin or cause any deep tissue damage, is sufficient to show if the animal is too light. Any observed movement (twitching of the skin) indicates that the animal is not sufficiently anesthetized to do surgery.

**Jaw "tone":** Generally this can be a good indicator of muscle relaxation. The lower jaw is gently opened to its maximum extent. Any observed resistance to opening, or closing of the mouth, is an indicator that the animal is too light to do a painful procedure.

**Respiratory rate:** This can be used as a good indicator of the depth (or level) of anesthesia. Rapid, shallow respirations usually indicate the animal is too "light," not sufficiently anesthetized to perform painful procedures. A very slow relaxed respiration may be an indication the animal is very 'deep,' even approaching euthanasia. Animals in deep anesthesia often take a very long time to recover, and will require additional supportive care during recovery. The best respiratory rate is one that is just barely below normal. Since normal respiration rate varies among animals, it is always important to observe your patient for a few minutes while they are resting to determine their normal respiration rate. Veterinary textbooks can provide a range of normal respirations, but even these vary from one animal to another. If obtaining a heart rate is difficult, then remember this: **A dead animal does not breath,** which is a simplistic way to say that if the animal is breathing then most assuredly the heart is beating.

**Heart rate:** An increase in heart rate and/or blood pressure usually indicates a decrease in anesthetic depth. Normal heart rates vary greatly among species, consult veterinary text for normal values, or Email our clinical veterinarian.

**Palpebral:** The blink reflex is quite variable, depending on the anesthetic agent used, and difficult to assess in small animals (i.e. mice and rats). **DO NOT USE THIS REFLEX FOR RODENT ANESTHESIA MONITORING!** It is highly UN-reliable!

**Corneal:** The cornea can be damaged, if not protected, but when used carefully, it is a good reflex. Touch the edge of the cornea with a gauge sponge or cotton q-tip. Movement of the eyelids is an indication that the depth of anesthesia is not sufficient to do surgery.

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**Body Temp:** Most anesthetic agents depress body temperature to a significant degree. Therefore, it is important that anesthetized animals be maintained on some type of material which shields them from contact with cold surfaces and reduces heat loss. The use of a supplemental heat source is a good idea, but must be used with caution, since burns can occur from electric blankets or water bottles that are too hot.

**Anesthesia Recovery:** All animals recovering from anesthesia must be constantly attended until they have recovered their swallowing reflexes. As a general statement, animals must be observed and the observation must be recorded at least every 15 minutes. Observing recovering animals less frequently than every 15 minutes requires IACUC approval. Observations (and recording of the observations) must continue until the animal regains motor control. In the case of most animals, this is usually indicated by the animal starting to move around the cage and being able to stand and walk without falling. Observations may be recorded in a research notebook or a medical record notebook, but should be available for IACUC review.

**Support of the Recovering Patient:** Rodent post-procedural support, and the duration of recovery, will be shortened by keeping the animal warm. A heat lamp, Snuggle Safe or a heating pad may be used, but the animal should not be close too the heat lamp (they will get skin damage from being too close) nor should they be in direct contact with the heating pad (the heating wire will also burn the animal). Usually, the best approach is placing the animal's cage half on and half off a heating pad, or wrapping the animal in a small towel placed in the bottom of the cage. Care must be taken to avoid overheating when a heating lamp or heating pad is used. Whenever a heat source is used, a thermometer should be placed at the animal's level to monitor actual heat.

Animals which have had any significant blood/fluid loss during surgery should be provided with fluid or blood replacement. In small rodents, this is best accomplished via the intraperitoneal or subcutaneous route and as described in the protocol.

## BUPRENORPHINE IS A GOOD CHOICE FOR RATS

In an article published in *Comparative Medicine* (2009 Feb;59(1):60-71), rats were evaluated for the effectiveness of Buprenorphine in a postoperative pain model (paw incision) in rats. The authors assessed acute postoperative pain relief, rebound hyperalgesia, and the long-term effects of postoperative opioid treatment on subsequent opioid exposure. Pain sensitivity to noxious and nonnoxious mechanical stimuli at the site of injury (primary pain) was assessed at 1, 4, 24, and 72 hours after surgery. Pain sensitivity at a site distal to the injury (secondary pain) was assessed at 24 and 72 hours after surgery. Rats were tested for their sensitivity to the analgesic and locomotor effects of morphine 9 to 10 days after surgery.

Buprenorphine dosed at 0.05 mg/kg and given subcutaneously was found to be the most effective of the tested agents. This dose induced isoalgesia during the acute postoperative period and the longest period of pain relief, and it did not induce long-term changes in opioid sensitivity in 2 functional measures of the opioid system.

**A lower dose of Buprenorphine** (0.01 mg/kg SC) did not meet the criterion for isoalgesia.

**A higher dose of Buprenorphine** (0.1 mg/kg SC) was less effective in pain relief at later recovery periods and induced a long-lasting opioid tolerance, indicating greater neural adaptations (long-term effects on opioid sensitivity).

### Upcoming Dates & Deadlines

November 2	New Protocol Deadline
November 5	Amendment Meeting
November 5	Mouse Methodology Workshop
November 12	Mouse Methodology Workshop
November 17	Mouse Genotyping Services Available From Transnetyx, Inc.
November 30	New Protocol Deadline
November 23	Amendment Deadline
January 4	New Protocol Deadline
January 7	Amendment Deadline

*Deadlines are 5 PM on the date listed!*

# A CHECKLIST FOR SUBMITTING ANIMAL USE APPLICATIONS (Obtaining approval to work with animals at Duke)

## GETTING STARTED:

- ◆ **VISIT THE DUKE ANIMAL PROGRAM WEB SITE** at: <http://vetmed.duhs.duke.edu/>
  - ◆ Familiarize yourself with this web site. This is the principle source for animal program forms, policies, guidelines, directions for training, or process procedures used in the Duke animal care and use program.
  - ◆ For more specific protocol development guidance click on the link at the left titled "Protocol Development." You may access the protocol template from this page, or by using the next step.
  - ◆ The protocol template may be found on the page ([http://vetmed.duhs.duke.edu/index\\_of\\_new\\_protocol.htm](http://vetmed.duhs.duke.edu/index_of_new_protocol.htm)).
    - ◆ Review the protocol template instructions.
    - ◆ Download the appropriate version on the template for your animal protocol (most researchers select 'Option A').
  - ◆ Visit the 'IACUC Meeting Deadlines' web page to confirm the review and approval schedule that best meets your needs ([http://vetmed.duhs.duke.edu/index\\_of\\_iacuc\\_meeting\\_deadlines.htm](http://vetmed.duhs.duke.edu/index_of_iacuc_meeting_deadlines.htm)). While most protocols are approved at the first IACUC meeting, you should plan on a 2 month approval process, just to be safe.
- ◆ **OBTAIN YOUR DUKE 'NetID' AND 'Password'**: While the animal program site is freely accessible, you will need your NetID and Password for certain steps toward protocol approval. NetID and Passwords are automatically created when you initiate a relationship with Duke (e.g. grad students' fees are paid at the registrar; faculty position acceptance letter has been received by Duke HR). If you have problems or don't know your NetID or Password, call the Office of Information Technology (OIT) at 919.684.2200.
- ◆ **COMPLETE THE HEALTH REVIEW**: The form for 'Health Review for Animal Handlers' is located at: <https://www.hr.duke.edu/secure/eohw/animal.php> Once submitted, the assessment usually takes a few days, but may take longer if EOHW (Employee Health) determines additional medical assessment or immunizations are necessary prior to working with animals, animal tissues, or animal byproducts.
- ◆ **COMPLETE THE BASIC WEB TRAINING FOR ANIMAL USERS**: Visit the OESO (safety office) web site at: <http://www.safety.duke.edu/>. Select the link on the left 'On-line Training.' Enter your NetID and Password. Select 'Courses Available On-Line.' All animal users must complete Animal Handlers I and Animal Handlers II. If you are working with rodents and will use CO2 for euthanasia, you must also complete 'CO2 Euthanasia of Rodents.' You may complete any of the other courses you feel are appropriate for your lab procedures. The IACUC, OESO, or EOHW may require specified web courses (e.g. biosafety work may require completion of the biosafety web module) during the protocol review.
- ◆ **FACILITY ORIENTATION**: Access to animal vivaria requires an approved protocol and Facility Orientation. Contact Peg Hogan, RLATG, CMAR (ph: 919.684.3885) or Email [hogan012@mc.duke.edu](mailto:hogan012@mc.duke.edu) for scheduling.

**PROTOCOL PRE-REVIEW:** To assist researchers with protocol development, the Office of Animal Welfare Assurance (OAWA) provides a pre-review service. Just complete your application for animal use as best you can, and Email it to [IACUC@DUKE.EDU](mailto:IACUC@DUKE.EDU). An OAWA veterinarian (Dr. Banks, Sharp, or Vanderford) will review your application and offer suggestions for improvement, in the hopes of achieving IACUC approval on the first review. This pre-review is not required, but it is the program default process and strongly encouraged to maximize an approval.

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## PROTOCOL SUBMISSION:

- ◆ **SUBMISSION FOR IACUC REVIEW:** When ready, submit all applications for animal use to [ICUC@DUKE.EDU](mailto:ICUC@DUKE.EDU). Once an application is submitted, DO NOT revise the document; only reply to the Emailed questions. Emails become part of the protocol file. *A REVISED APPLICATION WILL REQUIRE RE-REVIEW AND MAY DELAY PROTOCOL APPROVAL!*
  - ◆ **ADMINISTRATIVE REVIEW:** You will receive a confirmation from one of OAWA's Protocol Specialists that your protocol has been added to the IACUC's agenda. The Protocol Specialist will also perform an 'Administrative Review' and may suggest enhancements (e.g. missing contact information, missing housing or procedure location, etc.). Please reply by Email with the information requested.
  - ◆ **VETERINARY REVIEW:** All protocols receive a review by a Duke veterinarian prior to going to the IACUC. This review will include the entire protocol, but will focus on animal care, anesthesia, analgesia, and other animal care activities. The reviewer may identify specific concerns and offer suggestions to reconcile the concern. Please reply to the Email with the information requested. The pre-review and the veterinary review may occur at the same time, if both are performed by a veterinarian.
  - ◆ **OESO / EOHW REVIEW:** All protocols and amendments are reviewed by OESO and EOHW to assure compliance with Duke requirements for a safe workplace. You will receive an Email notice from OESO/EOHW concerning any issues that require attention. Please do not delay in responding to OESO/EOHW. Animal protocols may be reviewed by the IACUC, but the protocol approval will not be granted until PROTOCOL CLEARANCE has been received from OESO and EOHW.
  - ◆ **PRIMARY IACUC MEMBER REVIEWER:** A member of the IACUC will be assigned as the PRIMARY REVIEWER. A few days before the IACUC meeting you may receive an Email from the PRIMARY REVIEWER requesting clarification of certain points. Please reply to the Email with the information requested. The PRIMARY REVIEWER will be your advocate at the IACUC meeting.
- ◆ **NOTICE OF IACUC REVIEW:** Within 3 business days of the IACUC meeting you will be advised of the outcome of the Committee's review. If approved, you will be given a protocol registry number and instructions on annual reporting of your animal use activity. If additional clarifications are required to secure approval, you will receive a point-by-point breakdown of necessary actions to secure approval.

## PROTOCOL MAINTENANCE:

- ◆ **ANNUAL PROTOCOL REPORT:** A request for an annual report of your animal use activities will be sent via Email during the 10<sup>th</sup> month of the protocol. Please complete and return the annual report promptly, as IACUC approval is required by the 12 month anniversary, or the protocol may be suspended by the IACUC.
- ◆ **USDA REPORT:** The Duke animal program must report animal use to the USDA annually. A request for your animal numbers used will be sent via Email each fall. Please reply as soon as possible.
- ◆ **AMENDMENTS TO APPROVED ACTIVITY:** Any change of research direction, addition of new procedures or personnel, or changes in approved procedures must be IACUC approved prior to performing the changed activity. The Amendment form is available on the animal program website under 'FORMS AND REPORTS.' Depending upon the nature of the change, amendments may take between 3 and 14 business days for approval.
- ◆ **DE NOVO REVIEW:** According to NIH/PHS Policy, all protocols have a life cycle of 3 years. Projects continuing beyond 3 years must have a new protocol approved by the IACUC by the 36<sup>th</sup> month, or must be terminated. At the 33<sup>rd</sup> month of your protocol life cycle, OAWA will alert you by Email to submit a new protocol.
- ◆ **POST APPROVAL MONITORING:** Duke has an active program of Post Approval Monitoring (PAM). Compliance Liaisons will occasionally monitor animal procedures and confirm that the laboratory practices are as described in the approved protocol. Most laboratories will receive one visit per year, but depending upon the type of research, more monitoring sessions may be required. Researchers should view this process as partnering with the IACUC to assure program integrity. Deficiencies noted will be addressed quickly and in a collegial manner.